REMARKS

This "AMENDMENT AFTER FINAL REJECTION (Enclosed with RCE)" is made in compliance with 37 CFR 1.116.

Earlier, Examiner Fernandez expressed a willingness to possibly have an Interview after the Final Rejection. Although her willingness was greatly appreciated, because of a number of factors, including office clsoures and delays caused by the great snowfall of 2010, the Applicants did not attempt to schedule an Interview.

Nevertheless, this "AMENDMENT AFTER FINAL REJECTION (Enclosed with RCE)" is presented to clarify issues in going forward with the prosecution of this case under Continued Examination.

To avoid any possible confusion between their FIG. 2, their specification, and their claims, the Applicants point out in this "AMENDMENT AFTER FINAL REJECTION (Enclosed with RCE)", that the FIG. 2, the specification, and the claims are all consistent with each other.

As to why such amendments have not been presented earlier, Examiner Fernandez stated in her Office Action dated 08/19/2009, on page 8, the following: "Applicant's amendment necessitate the new ground(s) of rejection presented in this Office action".

These amendments are aimed at overcoming new ground(s) of rejection. Also, Examiner Fernandez said she may be willing to grant an Interview, and this option may be still possible in the future.

In her rejections of the Applicant's claimed invention, Examiner Fernandez has cited Hibi et al (4,800,163) and Meserol (5,720,921).

Here are key points that distinguish the Applicant's claimed invention from Hibi et al (4,800,163) and Meserol (5,720,921).

To improve the state of the art in the field of electroporation of biological materials, the Applicants have formulated FOUR IMPORTANT OBJECTIVES that are set forth within the four corners of the Applicants present patent application.

After years of experience (since before 1995) with developing methods and producing apparatuses for employing electroporation in the treatment of biological materials, and having already received a number of U. S. patents in the field of electroporation (e. g. U. S. Patent Nos. 6,010,613, 6,078,490, 6,603,998, 6,653,114, 6,713,291, 6,878,538, and 7,371,561), the

Applicants, working at Cyto Pulse Sciences, Inc., Glen Burnie, MD, have arrived at FOUR IMPORTANT OBJECTIVES to be attained by electroporation of biological vesicles and cells (biological materials) that are set forth within the four corners of the Applicants present patent application:

OBJECTIVE NO. 1: Control of heat in treating a suspension of biological materials in a static batch chamber with electroporation so that the biological materials are properly treated without being damaged by over heating.

OBJECTIVE NO. 2: Carrying out electroporation of a static batch of a suspension of biological materials in a chamber so that heating in the chamber is limited to low levels, so that the electroporation is carried out at room temperature without the need for cooling apparatus.

OBJECTIVE NO. 3: Treating large static volumes of suspensions of biological material at a time, that is 2 ml. or greater volumes of a biological material suspension in a chamber at a time, in a uniform manner, especially in a uniform manner with respect to electric fields.

OBJECTIVE NO. 4: Treating each static batch of a suspension of biological material in a uniform manner, especially in a uniform manner with respect to electric fields, so that sequentially treated batches cumulatively provide very large volumes of a uniform electroporation-treated biological product.

The Applicants claimed invention attains ALL FOUR of the OBJECTIVES stated above.

By providing a method as set forth in Claim 1 (and dependent claims) and an apparatus as set forth in claim 31 (and dependent claims), Applicants provide carrying out static electroporation of a suspension of biological materials in a chamber, wherein heating in the chamber is limited to low levels, to avoid damage to biological material (attaining OBJECTIVE NO. 1).

Because heating in the chamber is limited to low levels, the electroporation is carried out at room temperature, without using cooling apparatus (attaining OBJECTIVE NO. 2).

With the Applicants invention, a large static volume of a suspension of biological materials (of 2 ml. or greater) is treated by electroporation in a uniform manner, especially in a uniform manner with respect to electric fields, to provide a large batch of uniformly electroporation-treated biological material (attaining OBJECTIVE NOs. 3 and 4).

Also, sequential large batch volumes of suspensions of biological material are treated in a uniform manner, especially in a uniform manner with respect to electric fields, to produce cumulatively very large volumes of uniformly electroporation-

treated biological product (attaining OBJECTIVE NO. 4).

In sharp contrast, none of the Prior Art in the case, either alone or in combination, attains all four objectives. Actually, the Prior Art fails to attain OBJECTIVE NOs. 2, 3, and 4.

Hibi et al (4,800,163) FAIL to attain OBJECTIVE NOs. 2, 3, and 4.

For example, Hibi et al (4,800,163) do not operate at room temperature, and Hibi et al (4,800,163) use a cooling apparatus to operate at 0 deg. C (column 3, lines 49-51). Thus, Hibi et al (4,800,163) (FAIL to attain OBJECTIVE NO. 2)

In addition, Hibi et al (4,800,163) fail to provide large volume treatment of 2 ml. or greater (FAIL to attain OBJECTIVE NO. 3). More specifically, the volume of the chamber in Hibi et al (4,800,163) is only 50 microliters (column 6, lines 19-36).

Also, a failure to provide sequential batch electroporation treatment fails to provide cumulatively large amounts of uniformly treated electroporation-treated biological materials (FAIL to attain OBJECTIVE NO. 4).

Meserol (5,720,921) FAILS to attain OBJECTIVE NOs. 2, 3, and 4.

Turning to Meserol (5,720,921), this patent requires cooling

apparatus to handle the heating problem it creates with flow electroporation, and, as a result, does not operate at room temperature. Instead, Meserol (5,720,921) operates at between approximately 1 and 12 deg. C using a thermoelectric cooling coil 68 and a cooling reservoir 69 (from column 17, line 56 to column 18, line 13). Therefore, Meserol (5,720,921) (FAILS to attain OBJECTIVE NO. 2).

In addition, Meserol (5,720,921) uses flow electroporation, whereby, because of the nature of laminar flow and other flow-related issues with respect to treatment biological material suspensions with electric fields, the biological material is not treated uniformly, especially with respect to uniform treatment by electric fields. In this respect, Meserol (5,720,921) FAILS to attain OBJECTIVE NO. 4.

Moreover, Meserol (5,720,921) teaches away from the Applicants inventive way to attain OBJECTIVE NO. 4. That is, Meserol (5,720,921) states that heating problems are associated with continuous static electroporation treatments (column 6, lines 27-29). Meserol (5,720,921) offers no solution to the problem of heating with static electroporation treatments.

In addition, Meserol (5,720,921) FAILS to attain OBJECTIVE NO. 3 relating to large static volumes of biological material. Instead, the solution to the heating problem provided by Meserol (5,720,921) is to go in a completely different direction; that

is, flow electroporation, with all its attendant problems that are stated above briefly and that are described in the Applicants original specification in greater detail.

Summary

In summary, only the Applicant's claimed invention (NOT the Prior Art of Hibi et al (4,800,163) and/or Meserol (5,720,921)) attains all of the FOUR OBJECTIVES stated above, by solving the over heating problem of static batch electroporation of a suspension of biological materials in an electroporation chamber, so that heating in the chamber is limited to low levels, so that the electroporation is conducted at room temperature without the use of cooling apparatus.

With Hibi et al (4,800,163) and Meserol (5,720,921) FAILING to attain OBJECTIVE NOs. 2, 3, and 4 of the four objectives stated above, they should NOT prevent patenting of the Applicant's claimed invention which attains all four of the objectives.

In addition, in this REMARKS section, the following topics are addressed below in greater detail.

1. Significant Features of the Subject Invention
2. The Problem of Heating of Material Being Treated
3. Relating to the Specification
4. Relating to the Claims in General

1. Significant Features of the Subject Invention

During prosecution of the present patent application, in a need to answer the rejections and objections raised by Examiner Fernandez, some important features of the Applicants claimed invention may have not been emphasized enough. These important features were set forth in the original application, but during prosecution, were somehow not emphasized enough. Some of these important features of the present invention dramatically distinguish the invention from the Prior Art and from other participants in the present marketplace of electroporation methods and apparatuses. It should be emphasized that Cyto Pulse Sciences, Inc., Glen Burnie, Maryland, the employer of both of the present inventors, currently produces and markets methods and apparatus which embody the present invention.

First, in the Prior Art, there is no method or apparatus in electroporation which is carried out in statically retained

sequential batches (see original claim 19 and the original specification at page 21, lines 5-7). These characteristics, which embody the Applicants claimed invention, are embodied in the products of and the methods employing the products of Cyto Pulse Sciences, Inc..

Second, in the Prior Art, there is no static sequential method or apparatus in electroporation wherein a chamber has a chamber volume, the suspension has a suspension volume, and the suspension volume is greater than the chamber volume, and wherein

an initial portion of the suspension volume is moved into the chamber, retained and treated in the chamber, and moved out from the chamber, and

an additional portion of the suspension volume is moved into the chamber, statically retained and treated in the chamber, and moved out from the chamber (see original claim 28). This feature allows for great scalability. These characteristics, which embody the Applicants claimed invention, are embodied in the products of Cyto Pulse Sciences, Inc..

Third, in the Prior Art, there is no method or apparatus in electroporation which allows for repeated cycles set forth in original claim 28 (see original claims 29 and 30 and original specification at page 21, lines 17-25). These features provide further great scalabilty. These characteristics, which embody the Applicants claimed invention, are embodied in the products of

and the methods employing the products of Cyto Pulse Sciences, Inc..

Fourth, in the Prior Art, there is no method or apparatus in electroporation wherein the repetitive cycle of an first quantity of suspension is introduced in the chamber, is statically retained in the chamber for a selected period of time, is uniformly treated with complex pulsed electric field waveforms (see original claim 16 and the original specification spanning from page 20, line 27 to page 21, line 2) while being statically retained in the chamber, is removed from the chamber after such treatment, and is replaced by a second quantity of suspension which undergoes similar statically retained treatment with complex pulsed electric field waveforms while statically retained in the chamber prior to being removed from the chamber and replaced by a third quantity of suspension (see original claims 28, 29, and 30 and in the original specification at page 21, lines 17-30). This cycle can be repeated up to an Nth quantity, which provides for great scalabilty. These characteristics, which embody the Applicants claimed invention, are embodied in the products of and the methods employing the products of Cyto Pulse Sciences, Inc..

Fifth, in the Prior Art, there is no method or apparatus in electroporation which discloses the Applicant-defined Geometric Factor (GF) (see original claim 1 and currently amended claim 1,

and see original specification at page 25, lines 5-24). These characteristics, which embody the Applicants claimed invention, are embodied in the products of and the methods employing the products of Cyto Pulse Sciences, Inc..

Sixth, in the Prior Art, there is no method or apparatus in the art of electroporation in which chamber size and suspension characteristics are provided by adherence to the parameters of Conductivity, of the Applicant-defined Geometric Factor (GF), and of the electrical resistance in a region INSIDE THE TRIANGLE in the Applicants original FIG. 2. It is noted that this feature of the Applicants claimed invention is set forth in the two independent claims 1 and 31. Thus, all of the features of the Applicants claimed invention are dependent from this feature of the Applicants claimed invention. These characteristics, which embody the Applicants claimed invention, are embodied in the methods employing the products of Cyto Pulse Sciences, Inc..

Seventh, in the Prior Art, there is no method or apparatus in electroporation in which a chamber size of at least 2 milliliters is provided and in which parameters of the chamber and the material being treated inside the chamber are in accordance with the Applicants original FIG. 2. These characteristics, which embody the Applicants claimed invention, are embodied in the products of and the methods employing the products of Cyto Pulse Sciences, Inc..

Eighth, in the Prior Art, there is no method or apparatus in electroporation in which a chamber size of at least 2 milliliters is provided and wherein heating within the treatment cell (the chamber) is limited to low levels (see the original specification at page 16, lines 20-21 and page 17, lines 34-37). THIS MEANS THAT THE ELECTROPORATION IS CONDUCTED AT ROOM TEMPERATURE, WITH NO NEED FOR ANY COOLING APPARATUS AND WITH NO NEED FOR LOWERING THE TEMPERATURE OF THE MATERIAL BEING TREATED TO LESS THAN ROOM TEMPERATURE. These characteristics, which embody the Applicants claimed invention, are embodied in the products of and the methods employing the products of Cyto Pulse Sciences, Inc.. .

Ninth, in the Prior Art, there is no method or apparatus in electroporation in which the electric fields used to treat the cell suspension can be complex (many different types of pulse parameters) since the cell suspension is not moving. The electric field on-times are not constrained by the flow rates in flow systems. These characteristics, which embody the Applicants claimed invention, are embodied in the products of and the methods employing the products of Cyto Pulse Sciences, Inc..

Tenth, in the Prior Art, there is no method or apparatus in electroporation in which the invention uses a static chamber with volumes greater than 2 milliliters to insure that all cells are subject to the same electric field intensity and direction and the density of the cells and treating material are uniform.

These characteristics, which embody the Applicants claimed invention, are embodied in the products of and the methods employing the products of Cyto Pulse Sciences, Inc..

Aside from the significant features set forth above, to avoid any possible confusion between their FIG. 2, their specification, and their claims, the Applicants would like to point out that in this AMENDMENT AFTER FINAL REJECTION (Enclosed with RCE), the FIG. 2, the specification, and the claims are all consistent with each other.

More specifically, FIG. 2 shows a triangle which has three lines, three points of line intersection, and an interior space inside the triangle. The Applicants have made it perfectly clear in their specification that their Applicants claimed invention resides <u>inside</u> the triangle. See the applicants original specification at page 23, lines 9-10 which states,

> "FIG. 2 is a graph illustrating the operating range of the method of the invention, inside the triangle...".

To be consistent with this statement, the specification and the claims are amended herein.

In the real world of electroporation, the interrelationships of the chamber volume, geometric factor, medium conductivity and electrical resistance in the chamber are important. These

variables are used to calculate the dimensions of the electroporation chamber (gap and area).

To illustrate a real world example, two commercial systems were developed using these relationships. Cyto Pulse's CytoLVT-S™ and CytoLVT-P™ systems are large volume electroporation systems using chambers developed according to the instant invention. Chamber volumes, chamber gap, chamber geometric factor and conductivities of medium associated with these chambers are shown in the following Table 1. In addition, these models of the Cyto Pulse systems, do not employ any cooling system. These Cyto Pulse systems OPERATE AT ROOM TEMPERATURE in accordance with the currently amended claim 1 language of "wherein heating in the chamber is limited to low levels".

In addition, the following "TABLE A" presents data which show that by following the principles of the invention and the disclosures in the specification, a chamber having a 20 ml. capacity can be employed with heating in the chamber being limited to low levels, such that the electroporation can be carried out at room temperature.

TABLE A

Chamb. Volume (ml)	Chamber gap (cm)	Geometric Factor	Medium Conductivity Millisiemen/cm	Resistance at maximum volume and conductivity (ohms)
3	0.4	0.053	4	14
7	0.6	0.051	4	14
11	0.4	0.014	1	14
20	0.6	0.018	1	14

Stated somewhat differently, the inventive concept for the instant invention is the use of low conductivity medium with an electroporation chamber having dimensions determined using the Applicants novel mathematical formula of an Applicant-defined Geometric Factor (GF). There are a number of advantages to this combination described in the specification. One is large volume electroporation for processing large numbers of cells. A volume over 2 ml is listed as a preferred volume for each cycle of processing.

It is the interaction of these variables that is important to the instant invention. Both medium (buffer) conductivity and Applicant-defined Geometric Factor (GF) are important. The triangle in FIG. 2 is defined by the interaction of these two

variables. Also resistance contributes to the triangle in FIG. 2.

In FIG. 2, several examples of the Prior Art are illustrated. These are <u>outside</u> of the triangle that defines the inside region of the instant invention.

<u>2. The Problem of Heating of Material Being Treated</u>

In their original specification, the Applicants discussed the PRIOR ART PROBLEM OF HEATING of biological cellular material undergoing electroporation in a chamber and that biological cells are destroyed thereby.

In sharp contrast, at page 16, lines 20-21 of the original specification, the Applicants STATE A DESIRABLE FEATURE OF THEIR INVENTION:

> "(4) limits heating within the treatment cell to low levels".

At page 17, lines 34-37 in the original specification the Applicants STATE THAT DESIRABLE FEATURE OF THEIR INVENTION:

> "Even another object of the present invention is to provide a large volume ex vivo electroporation method that limits heating within the treatment cell to low levels."

In this respect, the electroporation conducted with the Applicants claimed invention, of limiting heating within the treatment cell (the chamber) to low levels, is conducted at room temperature.

Interestingly, Examiner Fernandez has cited Meserol (5,720,921) which is directed to continuous flow electroporation (eletro-transfection). Meserol (5,720,921) teaches away from "static" electroporation. More specifically, in Meserol (5,720,921), from column 5, line 28 to column 6, line 36, Meserol (5,720,921) discusses static electroporation techniques. In discussing the unsuitability of static electroporation techniques, Meserol (5,720,921) states at column 6, lines 27-29,"Continuous use of a 'static' chamber results in over heating of the chamber and increased cell lysis". This over heating is a big problem. Meserol (5,720,921) offers no solution to this over heating problem in "static" chambers.

Even with his continuous flow (not static) process, Meserol (5,720,921) creates unwanted heat. His solution to the heating problem is set forth from column 17, line 56 to column 18, line 13 which describes a thermoelectric cooling coil 68, a cooled temperature of between approximately 1 and 12 deg. C, and a cooling reservoir 69.

Clearly, only the Applicants claimed invention avoids any problem of unwanted heat, even with scale-up to at least 2 ml. in

a static chamber, because, with the Applicants claimed invention, heating within the treatment cell (the chamber) is limited to low levels.

The Applicants do not need a thermoelectric cooling coil 68 of Meserol (5,720,921), a cooled temperature of between approximately 1 and 12 deg. C of Meserol (5,720,921), or a cooling reservoir 69 of Meserol (5,720,921).

3. Relating to the Specification

a. Examiner Fernandez states that new matter has been injected into the specification by the previous AMENDMENT filed 05/06/2009. More specifically, Examiner Fernandez states that the phrase "above 2 milliliters" is not supported by the original application. Respectfully, the Applicants disagree with Examiner Fernandez on this point.

More specifically, the following is stated in the original application at page 20, lines 1-2:

> "Preferably, the chamber has at least a 2 milliliter capacity."

Also, the following is stated in the original application at page 21, lines 31-34:

> "In accordance with another aspect of the invention, an

> electroporation apparatus is provided which includes a chamber which has a chamber volume of at least 2 milliliters."

In view of the above, the language "above 2 milliliters" is supported by the original specification and is not new matter.

b. Examiner Fernandez objects to language in the specification, based on the AMENDMENT filed 05/06/2009, which she concludes is in conflict with FIG. 2 (which stands in its original form). In answer to these objections, the Applicants would like to point out that the claimed parameters relating to FIG. 2 are INSIDE the triangle in FIG. 2. This interpretation of FIG. 2 is stated in the original specification as follows at page 23, lines 9-13:

> "FIG. 2 is a graph illustrating the operating range of the method of the invention, INSIDE THE TRIANGLE, and how the operating range of the invention is outside operating ranges of prior art electroporation methods, indicated by small blocks outside the triangle."
>
> [emphasis added]

Generally, the line segments and points of intersection of the triangle itself are not included in the language in the currently claimed invention.

More specifically, the horizontal line of the triangle in FIG. 2, intersects 1.00 microSiemens/cm on the vertical axis for Conductivity in microSiemens/cm. Please take judicial notice that 1.00 microSiemens/cm equals 0.001 milliSiemens/cm.

In addition, the top point of the triangle reaches the level of 100,000.00 microSiemens/cm. Please take judicial notice that 100,000.00 microSiemens/cm equals 100 milliSiemens/cm.

Therefore, with respect to Conductivity, in accordance with the principles of the invention, to be INSIDE the triangle, the chamber has conductivity in a range spanning greater than 0.001 to less than 100 milliSiemens/cm. This is language in currently amended claim 1, and this language is in the specification to be consistent with the claim language. Clearly, no new matter has been entered.

Turning to the Applicant-defined Geometric Factor (GF) in relation to the triangle in FIG. 2, the portions of the triangle which bound the Applicant-defined Geometric Factor are a low point of 0.000001 cm^{-1} and a high point of 0.100000 cm^{-1}. So, to be INSIDE the triangle, the Applicants currently claimed invention states, "wherein said geometric factor is less than ~~or equal to~~ 0.1 (cm^{-1}) and greater than 0.000001 (cm^{-1})".

In this respect, the specification is amended to be consistent with the original FIG. 2 and the original

specification which states that the FIG. 2 relates to what is INSIDE the triangle. More specifically, the specification is herein amended in the paragraph on page 27, spanning lines 14-22, to be consistent with the parameters of the invention to be INSIDE THE TRIANGLE of FIG. 2, as follows:

The preferred operating region of the present invention is then:

Cell diameter > 1 micrometer

Chamber volume > 2 milliliters

Conductivity of Material to be treated > 1 microSiemens/cm

Conductivity of Material to be treated < 100,000 microSiemens/cm

Total resistance of material to be treated in chamber > 1 ohm

Geometric Factor of Chamber < 0.1 cm-1

Therefore, no new matter has been injected into the application.

Turning to the issue of "greater than one ohm", a similar issue arises. The line segment which forms the hypotenuse of the triangle in FIG. 2 is clearly labelled "R=1" which clearly stands for one ohm. However, to be INSIDE the triangle, and not on the one ohm line, the original specification clearly indicates on page 27, lines 20-21, as follows:

"Total resistance of material to be treated

in the chamber is > 1 ohm".

Clearly, then, the further recitation that the resistance is greater than one ohm is not new matter.

c. Examiner Fernandez objected to an amendment in the specification, in the AMENDMENT filed 05/06/2009, objecting to a chamber volume of "2 milliliters" rather than "1 milliliter".

In response the Applicants would like to point out original claim 3 which stated:

> "3. The method of claim 1 wherein the chamber has at least a 2 milliliter capacity."

Also, the original specification states at page 20, lines 1-2 the following:

> "Preferably, the chamber has at least a 2 milliliter capacity."

Also, the original specification states at page 21, lines 31-34 the following:

> "In accordance with another aspect of the invention, an electroporation apparatus is provided which includes a chamber which has a chamber volume of at least 2 milliliters."

In view of the above, clearly the language "2 milliliters" is not new matter.

In view of the above, it is respectfully requested that Examiner Fernandez reconsider and withdraw her objections to "new matter" being injected into the specification.

In addition, in the specification, the Applicants have deleted language including "miniscule", "virtually eliminates heating", and "virtually eliminate heating" from the specification because the Applicants feel that this language is not scientific and should not be recited in the specification or claims.

4. Relating to the Claims in General

Claims 1, 2, 4-6, 8, 16, 19-22, 28, 29, 31, 35, 38, 39, and 42 are currently in the case.

Claims 3 and 45 are cancelled herein. The content of claim 3 is incorporated in currently amended independent claim 1, and the content of claim 45 is incorporated in currently amended independent claim 31.

Claims 2, 4-6, 8, 16, 19-22, 28, 29 depend from claim 1. Claims 35, 38, 39, and 42 depend from claim 31.

5. Relating to Amendments to Claims 1 and 31

Briefly, independent claims 1 and 31 are currently amended herein to provide claim language that

> makes it clear that recited parameters fall INSIDE the triangle in original FIG. 2, as stated in the original specification at page 23, lines 9-10 as follows: "FIG. 2 is a graph illustrating the operating range of the method of the invention, inside the triangle".

The amendments to claims 1 and 31 are fully consistent with remarks made above about the interpretation of FIG. 2 with respect to INSIDE the triangle in FIG. 2 and amendments in the specification with respect to Conductivity, Applicant-defined Geometric Factor, and resistance in ohms.

Moreover, the current amendments to claims 1 and 31 clearly avoid all of the known Prior Art in the case.

More specifically, currently amended claim 1 now incorporates the limitations of claim 3, and claim 3 is cancelled. In addition, claim 1 is further amended herein to clearly provide that the geometric factor and the conductivity clearly reside <u>inside the triangle shown in FIG. 2</u> as stated in the original specification at page 23, line 10. That is, the geometric factor is less than 0.1 (cm^{-1}) and greater than 0.000001 (cm^{-1}). Also, conductivity is in a range spanning greater than 0.001 to less than 100 milliSiemens/cm,

In addition, currently amended claim 1 includes the following language: wherein heating in the chamber is limited to low levels. This language is supported in the original specification at the following places:

page 16, lines 20-21 which state:

"(4) limits heating within the treatment cell to low levels"; and

page 17, lines 34-37 which state:

"Even another object of the present invention is to provide a large volume ex vivo electroporation method that limits heating within the treatment cell to low levels."

Currently amended claim 31 now incorporates the limitations of claim 45, and claim 45 is cancelled. In addition, claim 31 has been further amended in the manner of amending claim 1 to clearly provide that the geometric factor and the conductivity clearly reside inside the triangle shown in FIG. 2 as stated in the original specification at page 23, line 10. In addition, currently amended claim 31 includes the following limitation: wherein heating in the chamber is limited to low levels.

6. Relating to Rejections Based on 35 U.S.C. § 103 and Meserol (5,720,921)

Examiner Fernandez rejected the Applicants claimed invention based on 35 U.S.C. § 103 and Meserol (5,720,921). Examiner Fernandez correctly states some features of Meserol (5,720,921), but Examiner Fernandez ignores that Meserol (5,720,921) teaches away from using "static" methods, that Meserol (5,720,921) describes over heating with "static" methods, provides no solution to the over heating problem with "static methods, and provides a cooling apparatus to solve the problem of over heating with his continuous flow methods.

Meserol (5,720,921) relates to continuous flow. The specification, drawings, and claims relate to continuous flow. All of the electrical parameters referenced by Examiner Fernandez relate to the continuous flow system. Moreover, with such a continuous flow system, the on-time of the electric field must be highly synchronized with the flow rate (see Meserol at column 15, lines 22-24). This is IN SHARP CONTRAST IS THE APPLICANTS CLAIMED INVENTION wherein the material is static when the electric field is applied. The static status of the material in the chamber by the Applicants ensures UNIFORM ELECTRIC FIELD TREATMENT. Such uniform electric field treatment is not achieved in the continuous flow system of Meserol (5,720,921). In addition, the static batch allows a uniform application of complex electric field waveforms to be delivered, such as in

claim 16 of the Applicants claimed invention. Such uniform application of complex electric field waveforms is not possible with the continuous flow system of Meserol (5,720,921).

As a matter of interest, Meserol (5,720,921) is cited in the Applicants specification in TABLE 4 on page 11 and is discussed on page 14, lines 20-29 of the Applicants original specification. Some deficiencies of Meserol (5,720,921) are discussed and are in sharp contrast with benefits of the Applicants claimed invention. More specifically, the Applicants specification lists and discusses Prior Art in both flow electroporation and static electroporation. See TABLE 4 on page 11. It is important to note that the Prior Art discussed TABLE 4 does not disclose sequential batch electroporation as provided by the Applicants claimed invention (see original Claim 19).

Also, it is recalled from the above remarks herein relating to discussion of heat issues, Meserol (5,720,921) discloses the unsuitability of static electroporation techniques, and Meserol (5,720,921) states "over heating of the chamber" as a big problem (see column 6, line 28).

Even with his continuous flow (not static) process Meserol (5,720,921) creates unwanted heat. His solution to the heating problem is set forth from column 17, line 56 to column 18, line 13 which describes a thermoelectric cooling coil 68, a cooled temperature of between approximately 1 and 12 deg. C, and a

cooling reservoir 69.

Clearly, only the Applicants claimed invention avoids any problem of unwanted heat, even with scale up to at least 2 ml. in a static chamber, because the heat created in the chamber, with the Applicants claimed invention, is limited to low levels.

The Applicants do not need a thermoelectric cooling coil 68 of Meserol (5,720,921), a cooled temperature of between approximately 1 and 12 deg. C of Meserol (5,720,921), or a cooling reservoir 69 of Meserol (5,720,921).

With respect to Meserol (5,720,921), Examiner Fernandez did not discuss any of the features relating to over heating and the cooling apparatus and the low temperature operation disclosed by Meserol (5,720,921).

Turning to another aspect of Meserol (5,720,921), Meserol (5,720,921) discloses and claims a continuous apparatus and method for conducting electroporation. Among all the drawings and the approximately 1,876 lines (28 columns X 67 lines/column) in the specification and claims, in over 99.84% of those lines, only the continuous methods and apparatus are discussed. Only three lines in the approximately 1,876 lines in the Meserol specification and claims relate to "static" and "single discrete batches" at column 15, lines 5-7, as follows:

> "A conventional electroporation chamber may
> be used when the operation of the apparatus

is static, namely, when single discrete batches of cells are processed."

With the phrase "conventional electroporation chamber" in conjunction with the word "single" in the phrase "single discrete batches", in the Applicants view, Meserol (5,720,921) clearly means that a conventional electroporation chamber will be used once (singly) and replaced with another conventional electroporation chamber for another batch. This is clearly different from the Applicants claimed invention wherein a single electroporation chamber is filled and emptied in "sequential batches" as set forth in Applicants claim 19.

It appears that Examiner Fernandez is implying that the Applicants "sequential batches" in the field of electroporation is disclosed in Meserol (5,720,921). The Applicants disagree with this implication in two respects. First, Meserol (5,720,921) does not disclose sequential batches in the field of electroporation. Second, conventional electroporation chambers, such as found in many scientific supply house catalogs, are "disposable". For example, see Websites for BioRad, Invitrogen. Interestingly, Meserol (5,720,921) also prefers a "disposable" flow electroporation chamber (see Meserol, column 15, lines 13-14), which is similar to a disposable "conventional electroporation chamber".

This conventional "disposable" nature of conventional electroporation chambers is not even mentioned in the four corners of the Applicants original specification.

Moreover, the Applicants specifically teach the re-use of the electroporation chamber over and over again. See the Applicants original specification at page 28, lines 4-9 which states:

> "One aspect of the invention further increases capacity by alternately filling and emptying the electrode. In this manner, all desired properties are met during a specific treatment and the electrode can be re-used for subsequent treatments in an intermittent batch process."

Other aspects of Meserol (5,720,921) are worthy of discussion to clarify the departure of Meserol (5,720,921) from the Applicants claimed invention.

First, the fluid flow through the Meserol (5,720,921) chamber is continuous. See Column 9 lines 30 and 31 "thus it is an object of the invention to provide an automated continuous flow encapsulation apparatus." The phrase "continuous flow" appears several places in Meserol (5,720,921). Another example from Meserol (5,720,921) follows: "The present invention further comprises a new flow electroporation chamber that allows use of

the chamber under flow rather than static conditions". See Column 11, lines 6-8.

Static electroporation as discussed in the Meserol (5,720,921) patent is single batch (one fill) only. "A conventional electroporation chamber may be used when the operation of the apparatus is static, namely, when single discrete batches of cells are processed." See Column 15 lines 5-7.

In fact Meserol (5,720,921) teaches away from the use of static electroporation when used repeatedly, which is provided by the Applicants claimed invention. Meserol (5,720,921) states: "The electroporation methods disclosed in the prior art are not suitable for processing large volumes of sample, nor use of a high or repetitive electric charge. Furthermore, the methods are not suitable for use in a continuous or "flow" electroporation chamber. Available electroporation chambers are designed for static use only. Namely, processing of samples by batch. Continuous use of a "static" chamber results in over heating of the chamber and increased cell lysis." See Column 6, lines 21-28.

The heating that Meserol (5,720,921) refers to is Joule heating as a result of electrical current. That problem was solved in the Applicants claimed invention by controlling electrical resistance through the use of chambers designed using

the Applicant-defined Geometric Factor (GF) and low conductivity buffers, through use of parameters inside the triangle in FIG. 2 of the Applicants original drawing FIG. 2.

Meserol (5,720,921) does not mention the use of low conductivity buffer to limit current and heating. All buffers shown as examples in the Meserol (5,720,921) patent are high conductivity buffers.

In view of the above, Meserol (5,720,921) should be withdrawn as applied to the rejection of the Applicants claimed invention under 35 U.S.C. § 103.

7. Relating to Rejections Based on 35 U.S.C. § 103 and Hibi et al

Examiner Fernandez states that Hibi et al (4,800,163) disclose parameters of chamber volume and electrode gap, which when calculated in accordance with Applicant-defined Geometric Factor (GF) calculate to be less than 0.1 (cm^{-1}). As far as Examiner Fernandez's statement goes, this is true.

However, it should realized that Hibi et al (4,800,163) disclose two types of treatments of cellular material: electro-fusion and electro-transfection.

Hibi et al (4,800,163) discloses the following about electro-fusion at column 1, lines 13-27:

"Electro-fusion which was developed and

> established by Zimmermann et al in 1981 relies on the following principle. A cell suspension is introduced into a space between the electrodes disposed in parallel. When an alternating current is applied between the electrodes, the cells are moved in an electric field in the direction of the highest field strength and are oriented into a configuration resembling of a pearl chain. Application of direct-current pulses having a duration ranging between several .mu.s to several tens of .mu.s to the cells in this state induces reversible disorder of the lipid bilayers constituting the cell membranes and reassembly thereof, which leads to fusion of the cells (Angew. Chem. Int. Ed Engl. 20. 325-344, 1981; PHYSIOL. PLANT. 67:50 7-516. Copenhagan 1986)."

Also, Hibi et al (4,800,163) discloses the following about electro-transfection at column 1, lines 28-34:

> "Electro-transfection is a technique developed in 1982 by Neumann et al. This involves the introduction of a mixture of cells and nucleic acids between the

> electrodes and application of direct-current pulses having a duration ranging between several .mu.s to several tens of .mu.s between the electrodes which causes introduction (transfection) of the nucleic acids into the cells."

Clearly, only the disclosures relating to "electro-transfection" in Hibi et al (4,800,163) are relevant with respect to the Applicants claimed invention. The Applicants claimed invention is directed to electroporation which is clearly closely related to Hibi et al's electro-transfection, not electro-fusion.

However, for either electro-fusion or electro-transfection, Hibi et al (4,800,163) do not even discuss any of: the Applicant-defined "geometric factor"; conductivity of chamber contents; and the electrical resistance of chamber contents. That is, Hibi et al (4,800,163) do not even mention let alone discuss these key aspects of the Applicants claimed invention.

In view of these facts, it may be said that by using the Applicant-defined Geometric Factor (GF) and by using chamber and electrode gap parameters in Hibi et al (4,800,163), BY MERE COINCIDENCE, the calculation results in a Geometric Factor value that is less than 0.1 (cm^{-1}), which is specified in the Applicants claimed invention. However, even though this one fact is true, Hibi et al (4,800,163) is still silent as to the nature

of the Applicant-defined "geometric factor", is still silent as to the Applicant-defined calculation for the "geometric factor", is still silent as to conductivity in the chamber, and is still silent as to electrical resistance of the material in the chamber.

The only "factors" disclosed in Hibi et al (4,800,163) are disclosed from column 3 line 67 to column 4 line 17 which relates to electro-fusion, not electro-transfection, and which states: "While observing the waveform of the output of the oscillator 40 and pulse generator 42 by means of the oscilloscope 44, the frequency and amplitude of the output of the oscillator 40, the duration and voltage of the output pulses of the pulse generator 42, and the number of pulses generated by the pulse generator 42 during one operation are manually and suitably set in accordance with the thickness of the spacer 24, the type of sample and the flow rate set. If the cells employed are plant protoplasts, these factors are set at the following values: the frequency and the strength of the electric field generated between the electrodes by the oscillator 40 are set at values ranging between 0.5 and 2 MHz and between 100 and 500 V.sub.p-p /cm, respectively. The duration, strength of the electric field and intervals between the pulses generated from the pulse generator 42 range between 10 .mu.s and 1 ms, between 0.5 and 2 kV/cm, and between 10 ms and 1 s, respectively."

Clearly, these "factors" disclosed in Hibi et al (4,800,163) do not relate to the Applicant-defined Geometric Factor (GF), conductivity, or resistance in ohms.

Moreover, the word "conductivity" and the word "ohm" are not found in the four corners of Hibi et al (4,800,163).

In contrast, key aspects of the Applicants claimed invention are set forth graphically in the Applicants original FIG. 2. Hibi et al (4,800,163) does not provide any teaching that even remotely approximates the Applicants disclosures in FIG. 2. The silence of Hibi et al (4,800,163) in these key aspects of the Applicants claimed invention should not permit Hibi et al (4,800,163) (either alone or in combination with Meserol (5,720,921)) to block the allowance of the Applicants claimed invention.

Furthermore, neither Hibi et al (4,800,163) nor Meserol (5,720,921) teach a formula or graphical method of scaling up. Of course, a person of ordinary skill in the art could easily take the Hibi et al (4,800,163) chamber and scale it up in volume to be used in the Meserol (5,720,921) method. HOWEVER, WHAT IS MISSING IN HIBI ET AL (4,800,163) AND MESEROL (5,720,921) ARE MEANS TO SCALE-UP THE VOLUME WITHOUT THE UNDESIRABLE EFFECT OF OVER HEATING.

Hibi et al (4,800,163) recognize the problem of over heating and their solution to the problem is to conduct the electro-

transfection at 0 deg. C ((column 3, lines 49-51). As stated above Meserol (5,720,921) handles the problem of over heating during electroporation by employing a cooling apparatus and conducing electroporation at a cooled temperature of between approximately 1 and 12 deg. C.

Both Hibi et al (4,800,163) and Meserol (5,720,921) cannot carry out electro-transfection or electroporation at room temperature because the parameters they employ cause over heating. This is in sharp contrast with the Applicants claimed invention in which electroporation is carried out at room temperature because heating in the chamber is limited to low levels.

Also, Hibi et al (4,800,163) teach continuous flow with electro-transfection and continuous flow with static cell fusion (Col 1, lines 50-55), as follows:

> "Accordingly, an object of the present invention is to provide a flow chamber and an electro-manipulator incorporating the same which can be operated easily, and which are capable of continuously conducting electro-fusion or electro-transfection on a large amount of cells."

Moreover, details disclosed for electro-transfection examples in Hibi et al (4,800,163) are clearly for a continuous

(not static batch) process. More specifically, Hibi et al (4,800,163) state the following at column 6, lines 19-36 which states:

> "A test example of this transfection of nucleic acids will now be described. The same electro-manipulator as that described above was used, with the exception of the flow chamber. The flow chamber used had a volume of 50 .mu.l and an inter-electrode distance of 0.5 mm. Tobacco mesophyll protoplasts (cv: Xanthi NN) which were suspended at a concentration of approximately 2.times.10.sup.5 cells/ml in 0.5 M-mannitol with 100 .mu.M MgCl.sub.2 and which were mixed with tobacco mosaic virus (TMV) RNA at a concentration of 10 .mu.g/ml, were employed as the sample. Pulses having a height of 40 V and a duration of 50 .mu.s were applied at a frequency of 10 cycles/s to this sample while <u>it was being continuously fed by the suction pump 50 at a rate of 50 .mu.l/s</u> [emphasis added]. This operation was continued for 80 seconds, until a total of 4 ml or 8.times.10.sup.5 of protoplasts were

obtained. The transfection rate of the nucleic acids and survival rate of the cells were 96% and 95%, respectively."

This continuous flow method of Hibi et al (4,800,163) is in sharp contrast with the Applicants invention of static batch electroporation.

Turning to another issue, Examiner Fernandez correctly points out that there is one chamber of Hibi et al (4,800,163) wherein if the formula of the instant invention were used to calculate its geometric factor, the value would be 0.05 which is inside the triangle in FIG. 2. Hibi et al (4,800,163) illustrates that it is possible to randomly design a chamber with a geometric factor within the boundary of the instant invention. HOWEVER, SINCE THE APPLICANT-DEFINED GEOMETRIC FACTOR (GF) WOULD BE UNKNOWN TO THE DESIGNER, IT WOULD NOT BE POSSIBLE TO SCALE UP THE DESIGN AND MAINTAIN A GEOMETRIC FACTOR WITHIN THE INSTANT INVENTION WITHOUT THE FORMULA OF THE INSTANT INVENTION. As pointed out above, Hibi et al (4,800,163) offers no teaching as to what design principles were used in providing the continuous flow chamber used for electro-transfection.

For example, the Hibi et al (4,800,163) chamber has a 50 µL capacity with a 0.5 mm or 0.05 cm gap. From this, the area can be calculated (Length X Width). Without the geometric factor, some means must be used to scale-up the volume. There is no

mention of how this would be done in the Prior Art because scale-up is seldom mentioned. There is no mention in the literature of a means to scale-up a chamber of any given dimension.

To the Applicants knowledge, there are no examples of chambers over 2 ml in capacity with dimensions that could be retroactively calculated to have a geometric factor less than 0.1 $(cm)^{-1}$.

In spite of the discussed deficiencies in the disclosures of Hibi et al (4,800,163) and Meserol (5,720,921), Examiner Fernandez concludes that it would be obvious for a person of ordinary skill in the art to use the Applicant-defined Geometric Factor (GF) calculated from Hibi et al (4,800,163) to be adapted to the system of Meserol (5,720,921). Examiner Fernandez states that "routine experimentation" could adapt the Applicant-defined Geometric Factor (GF) in Meserol (5,720,921) to the Applicant-defined Geometric Factor (GF) of Hibi et al (4,800,163). Therefore, Examiner Fernandez concludes, the Applicants claimed invention would be rendered obvious under 35 U.S.C. § 103.

Respectfully, Examiner Fernandez uses improper reasoning for a number of reasons.

First, is her use of the Applicant-defined

Geometric Factor (GF) in the calculation involving the Prior Art of Hibi et al (4,800,163). This calculation involves using

inter-electrode gap and cell volume. In fact, the only calculation in Hibi et al (4,800,163) relates to a calculation of a time in minutes. At column 5, lines 21-22 Hibi et al (4,800,163) state the following: "A total of 40 minutes (equal to 2 minutes per cycle X 20 times) was required to complete the test."

Second, is her allegation that the same Applicant-defined Geometric Factor (GF) could be used by a person of ordinary skill in the art to adapt the system of Meserol (5,720,921) to the conditions of the Applicants claimed invention.

Moreover, nowhere is the Applicant-defined Geometric Factor (GF) found in the Prior Art. Therefore, a person of ordinary skill in the art could not use the Applicant-defined Geometric Factor (GF). It is simply not proper for any Examiner to allege that a person of ordinary skill in the art could simply derive the Applicant-defined Geometric Factor (GF) simply by using ordinary skill. No demonstration of that hypothesis has been or could be offerred by Examiner Fernandez.

Moreover, if the Applicant-defined Geometric Factor (GF) is in the Prior Art for the field of electroporation, the Applicants would surely like to know about it. Examiner Fernandez has certainly not found it in the Prior Art. However, no such

Applicant-defined Geometric Factor (GF) exists in the Prior Art to the knowledge and belief of the Applicants.

In view of the above, it is respectfully requested that Examiner Fernandez withdraw the rejections of the Applicants claimed invention based on Meserol (5,720,921) and Hibi et al (4,800,163) under 35 U.S.C. § 103.

8. Comments Relating to "Response to Arguments"

In the "Response to Arguments" portion of the Office Action dated 08/19/2009, Examiner Fernandez makes an outlandish assertion. On the one hand, she concedes that Meserol (5,720,921) and Hibi et al (4,800,163) do not define the "geometric factor". Yet, on the other hand, she asserts that the geometric factor exists given that there is an electrode gap and a chamber volume.

In addition, Examiner Fernandez states, "While Meserol does not teach the recited geometric factor, it is respectfully noted that Hibi et al. teaches this aspect of the claimed invention. Moreover, varying the volume, the electrode distance, and solution conductivity would have been a matter of routine experimentation."

In rebuttal, respectfully, the Applicants would like to point out that Hibi et al (4,800,163) DO NOT TEACH THE GEOMETRIC

FACTOR ASPECT OF THE CLAIMED INVENTION.

As stated above, the only "factors" disclosed in Hibi et al (4,800,163) are disclosed from column 3 line 67 to column 4 line 17 which relates to electro-fusion, not electro-transfection, and which states:

> "While observing the waveform of the output of the oscillator 40 and pulse generator 42 by means of the oscilloscope 44, the frequency and amplitude of the output of the oscillator 40, the duration and voltage of the output pulses of the pulse generator 42, and the number of pulses generated by the pulse generator 42 during one operation are manually and suitably set in accordance with the thickness of the spacer 24, the type of sample and the flow rate set. If the cells employed are plant protoplasts, these factors are set at the following values: the frequency and the strength of the electric field generated between the electrodes by the oscillator 40 are set at values ranging between 0.5 and 2 MHz and between 100 and 500 V.sub.p-p /cm, respectively. The duration, strength of the electric field and intervals

between the pulses generated from the pulse generator 42 range between 10 .mu.s and 1 ms, between 0.5 and 2 kV/cm, and between 10 ms and 1 s, respectively."

Clearly, these "factors" disclosed in Hibi et al (4,800,163) do not relate to the Applicant-defined Geometric Factor (GF), conductivity, or resistance in ohms, as recited in the Applicants claimed invention.

With respect to Hibi et al (4,800,163), Examiner Fernandez states that "solution conductivity would have been a manner of routine experimentation". In fact, the word "conductivity" and the word "ohm" are not found in the four corners of Hibi et al (4,800,163). Therefore, it defies reason that Hibi et al (4,800,163) would conduct routine experimentation on two essential parameters of the Applicants claimed invention when Hibi et al (4,800,163) does not even consider "conductivity" and "ohm" in the four corners of Hibi et al (4,800,163).

The Applicants would like to remind Examiner Fernandez that the Applicants are setting forth is their Applicant-defined Geometric Factor (GF), not just any geometic factor. Their Applicant-defined Geometric Factor (GF) provides the following ratio: GF = $(gap)^2$/volume. Please see the formula in the Applicants original specification on page 25, lines 5-17. In this respect, (1/sigma) X $(l)^2/v$ = GF/sigma

By simply multiplying both sides of the equation by sigma, the GF = $(l)^2/v$

Please recall that in the Remarks section of the AMENDMENT filed on May 6, 2009 by the Applicant's representative, the following was stated:

> "More specifically, on May 4, 2009, the term "geometric factor" was searched on the Google search engine with the following search statement: "geometric factor" electroporation chamber. There were 96 hits, and there were 38 hits actually displayed. All of the displayed 38 hits were looked at, and only references to the Applicants claimed invention, by way of the Applicants U. S. Patent Application Publication No. US 2006/0108229 A1, was there a discussion of a "geometric factor" that relates to the Applicant-defined "geometric factor". Once again, clearly the Applicant-defined "geometric factor" is not part of the Prior Art."

It would have been impossible for a person of ordinary skill in the art, prior to the filing date of the Applicants PCT International Patent Application on March 15, 2004, to read

Meserol (5,720,921) which was published on February 24, 1998, to read Hibi et al (4,800,163) which was published on January 24, 1989, and to use the Applicant-defined Geometric Factor (GF) in any calculation prior to March 15, 2004. Therefore, discussing the Applicant-defined Geometric Factor (GF) and the Prior Art of Meserol (5,720,921) and Hibi et al (4,800,163) is not proper.

Also, in the "Response to Arguments" portion of the Office Action dated 08/19/2009, Examiner Fernandez states that the Applicants claimed invention fails to speak of "scaling up". Although the exact phrase "scaling up" is not used in the Applicants original specification and claims, the concept of "scaling up" is expressed in other words. More specifically, in the original specification, at page 16, lines 15-17, the Applicants state:

> "(2) is scalable so that substantially large volumes of ex vivo or in vitro cells can be processed in a relatively short period of time;".

Also, in the original specification, at page 29, lines 24-27, the Applicants state:

> "With the invention, a large volume ex vivo electroporation method is provided which is

scalable so that substantially large volumes of ex vivo or in vitro cells can be processed in a relatively short period of time."

Also, in the original claim 1, which has since been amended several times without losing the "scalable" feature, at page 32, in lines 20-21 of original claim 1, the Applicants state:

"the treatment volume of the suspension is scalable".

Clearly, then, Examiner Fernandez's assertion that "scaling up" is not in the claims is mistaken.

Also, in the "Response to Arguments" portion of the Office Action dated 08/19/2009, Examiner Fernandez states that the Applicants claimed invention fails to speak of temperature. Examiner Fernandez is correct that the Applicants claims do not recite the word "temperature". However, the Applicant's claimed invention does address temperature by their recitation of "<u>wherein heating in the chamber is limited to low levels</u>" in claims 1 and 31.

In addition, at page 21, lines 3-4 in the original specification, there was a direct mention of "temperature", in which the Applicants stated:

"With the method of the invention, the

temperature rise during vesicle treatment is miniscule."

In the current "AMENDMENT AFTER FINAL REJECTION (Enclosed with RCE)", it is noted however, that the just-quoted sentence, having the word "miniscule" therein, is deleted from the specification because the Applicants feel that the word "miniscule" is not scientific and should not be recited in either the specification or a claim. In addition, the reference to "temperature" is deleted from the specification because the Applicants feel they have not clearly defined it in the specification.

However, a closely related concept is still present in the specification in the form of "limits heating within the treatment cell to low levels" and is present in the currently amended claims in the equivalent form of "wherein heating in the chamber is limited to low levels". In that sense Applicants currently address the concept of temperature in the claims (in the context of the temperature problems discussed in Meserol (5,720,921) and in Hibi et al (4,800,163)) without directly reciting the word "temperature" per se.

In view of the above, it is respectfully requested that Examiner Fernandez withdraw her rejections of the Applicants claimed invention based on Meserol (5,720,921) and Hibi et al (4,800,163).

9. Closing Remarks

In addition to the ten significant features of the Applicants claimed invention set forth in the above section "1. Significant Features of the Subject Invention", the following significant points have been set forth above.

None of the Prior Art discloses an electroporation chamber having a suspension of biological materials therein that has the following features:

(a) is greater than 2 milliliters in volume,

(b) has an Applicant-defined Geometric Factor (cm^{-1}) defined by the quotient of the electrode gap squared (cm^2) divided by the chamber volume (cm^3),

(c) wherein said geometric factor is less than 0.1 (cm^{-1}) and greater than 0.000001 (cm^{-1}),

(d) has a suspension in the treatment volume in the chamber having conductivity in a range spanning greater than 0.001 to less than 100 milliSiemens/cm,

(e) wherein the resistance of the suspension in the chamber is greater than one ohm,

(f) wherein heating in the chamber is limited to low levels, such that the treatment in the chamber is carried out at room temperature,

(g) wherein the treatment volume of the suspension is

scalable from 2 milliliters to above, and

(h) the parameters of Applicant-defined Geometric Factor (GF) of the chamber, and conductivity and resistance of the suspension are inside the triangular region shown in FIG. 2, as set forth in currently amended claims 1 and 31.

In view of the above, it is respectfully requested that the Examiner reconsider the rejections of the Applicants claimed invention in view of Meserol (5,720,921) and/or Hibi et al (4,800,163) and allow the following claims of the Applicants which are currently in the case:

Claims 1 (Currently amended), 2, 4-6, 8, 16, 19-22, 28 (Currently amended), 29, 31 (Currently amended), 35, 38, 39, and 42.

On the basis of the above amendment and remarks, reconsideration of the application is requested.

It appears that all matters have been addressed satisfactorily, and that the case is now in condition for a complete allowance; and the same is respectfully urged.

In view of the foregoing, it is respectfully requested that claims 1, 2, 4-6, 8, 16, 18, 19-22, 28, 29, 31, 35, 38, 39, and 42 be deemed allowable. If the Examiner believes otherwise, or has any comments or questions, or has any suggestions for putting the case in condition for final allowance, the Examiner is respectfully urged to contact the undersigned attorney of record

at the telephone number below, so that an expeditious resolution may be effected and the case passed to issue promptly.

Respectfully submitted,

02/18/2010
Date

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